IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	MARECHAL E., *et al.*	) Group Art Unit: 1645
Serial N°:	09/926,169	) Examiner: Tammy K. FIELD
Filed:	September 18, 2001	) Attorney's Docket N°213993US0PCT
For:	SCREENING METHOD INVOLVING MGDG SYNTHASE	)

DECLARATION UNDER 37 C.F.R.§ 1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Eric MARECHAL, hereby declare and state as follows:

1. I am Doctor of Science in Cell Biology and a co-Applicant of the Patent Application N° 09/926,169 filed on September 18, 2001,

2. I have been working as a researcher in a Vegetal Cell Physiology Laboratory associated between the University Joseph Fourier in Grenoble (France) and the "Commissariat à l'Energie Atomique" also in Grenoble since 1998, after several years of research at the Rockefeller University (New York, USA),

3. I have read and understood the Office Action of November 28, 2003 and the prior art cited by the Examiner,

4. I have specifically read the comments of the Examiner about the lack of clarity of the Patent Application regarding the relationship between screening and selecting antiparasitic agents and/or herbicides and a MGDG synthase inhibitor for use in a pharmaceutical composition for treating an animal having an apicomplex parasite and/or use as herbicide,

5. Under my supervision, the following study has been performed:

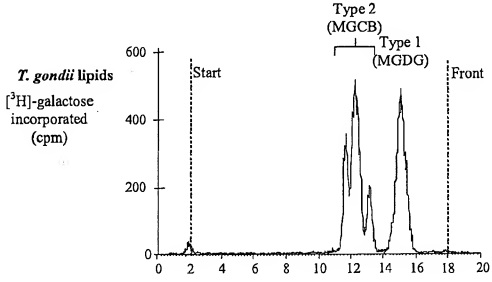
2×10^8 cells of *Toxoplasma gondii*, in the form of tachyzoites, have been suspended in 0.1 ml of a 1:10 mixture of 10 mM 3-(*N*-morpholino)propane sulfonic acid (MOPS), pH 7.8 and 1 mM dithiothreitol (DTT), containing 2% (w/v) glycerol and 50 mM of potassium chloride, and then incubated for 30 minutes in the presence of 4 µCi of UDP-[^{3}H]-galactose (7.5 nmol).

The glycolipids have been extracted according to the method of Bligh and Dyer, (Can. J. Biochem. Physiol., 1959, **37**, 911-917), then analyzed by thin layer chromatography (TLC) on 60 µ-silica gel plates resolved with a 65/25/4 (v/v) chloroform/methanol/water mixture,

in the presence of control lipids (MGDG; bovine brain monogalactosyl cerebroside (MGCB); digalactosyldiacylglycerol (DGDG); trigalactosyldiacylglycerol (triGDG) and tetragalactosyldiacylglycerol (tetraGDG)).

The radioactivity of the labeled lipid has then been detected using a TLC-analyzer device (LB2842 automatic TLC scanner).

The results obtained are given in the figure below, which illustrates the amount of labeled galactose (cpm) incorporated by the *Toxoplasma gondii* cells as a function of migration in centimeters.



On this figure, it is possible to see three first peaks which migrate to the same degree as the MGCB, whereas the last peak migrates to the same degree as the MGDG.

After migration, the lipids have been visualized by spraying, onto the silica gel plates, a solution comprising 0.2% of orcinol and 75% of sulfuric acid, and then heating at a temperature of 100°C for 15 minutes (results not shown).

The peak corresponding to the MGDG disappeared after alkali hydrolysis for 3 hours with 0.1 N potassium hydroxide in a water/methanol mixture.

Complete identification of the lipids has been carried out after hydrolysis of the polar head with α-galactosidase from green coffee beans and β-galactosidase from bovine testes, and deacylation by alkali hydrolysis under gentle conditions (0.1 N potassium hydroxide in a water/ethanol mixture for 3 hours).

Peak 4 was found sensitive to hydrolysis with β-galactosidase, which shows that the galactose is clearly linked in the beta position, as for MGDG.

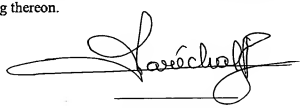
Moreover, after alkali hydrolysis, peak 4 disappeared, which demonstrates that the lipid present in the *Toxoplasma gondii* membrane clearly contains half diacylglycerol.

Conclusion

This study clearly demonstrates the presence of MGDG in an apicomplex parasite, *Toxoplasma gondii* and that, consequently, the MGDG synthase which serves as a target to search for a molecule with antiparasitic properties according to the Invention disclosed in US patent application n°09/926,169 clearly exists in apicomplex parasites.

Therefore, this study confirms that one of the possible applications of the search for inhibitors of plant MGDG synthase is the identification of anti-apicomplex parasite agents.

6. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of this Application or any Patent issuing thereon.



Eric MARECHAL

Date 2004, march 18th